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The physical state of the LDL core influences the conformation of apolipoprotein B-100 on the lipoprotein surface

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Abstract **We assessed the influence of temperature on the secondary structure of apolipoprotein B-100 (apoB) in normal low-density lipoprotein (N-LDL) and triglyceride-rich LDL (T-LDL). Gradual heating from 7°C to the phase-transition temperature of the lipoprotein core (~28°C and ~15°C for N-LDL and T-LDL, respectively) gradually altered the secondary structure of apoB, while further heating from the phase-transition temperature to 45°C had no additional effect. Above the phase-transition temperature of the core, the apoBs of N-LDL and T-LDL had a similar secondary structure. These results indicate that the conformation of apoB on the LDL surface depends strongly on the physical state of the lipoprotein core, and less on the lipid composition of the core per se.**

Key words: Cholesteryl ester; Liquid state; Liquid-crystalline state; Differential scanning calorimetry; Circular dichroism spectroscopy

1. Introduction

The low-density lipoprotein (LDL) plays a critical role in human cholesterol metabolism, and elevated plasma levels of LDL are associated with increased risk of heart disease and stroke [1,2]. The metabolic and pathogenic properties of LDL depend on the structure of apolipoprotein B-100 (apoB), which is the only apoprotein of LDL [3]. However, information on the detailed structure–function relationships of apoB is currently limited by a lack of high-resolution structural data.

ApoB is a large glycoprotein molecule (M_r ca. 550 000) that is thought to form a ring around the hydrophobic core of the LDL particle [4,5]. The deduced amino acid sequence of apoB indicates that the molecule contains five alternating domains of amphipathic α-helical and β-sheet structures ($\beta\alpha_1$–β_1–α_2–β_2–α_3) [3]. The N-terminal $\beta\alpha_1$ domain, partly modeled after the X-ray crystal structure of lamprey lipovitelin [6], plays a critical role in the co-translational assembly of lipoprotein particles in the liver [3,7,8]. The C-terminal 11% of apoB, including the α_3 domain, forms a 'bow' structure that functions as a negative modulator of LDL-receptor binding [5,9,10].

Studies have indicated that the conformation of apoB depends on the lipid content of the lipoprotein particle. For example, the folding of apoB changes during the conversion of very-low-density lipoprotein to LDL [9–11], and the conformation of apoB in small dense LDL differs from the conformation of apoB in larger, more buoyant LDL particles [12,13]. Thus, the size and lipid composition of the LDL core appear to influence the structure of apoB on the lipoprotein surface.

Recent studies indicate that the conformation of apoB may also depend on the physical state of the LDL core. While native LDL with a liquid core is generally thought to be spherical, cryo-electron microscopic studies suggest that the lipoprotein particle has an oblate ellipsoid or discoid shape when its core is in the liquid-crystalline state [14–17]. In addition, dietary studies with non-human primates indicate that LDL particles with a liquid-crystalline core have a higher affinity for vascular proteoglycans than LDL particles with a liquid core [18,19]. However, a direct association between changes in the physical state of the core and changes in the conformation of apoB remains to be established.

In the present study, we correlated the phase-transition of the LDL core with the changes in the secondary structure of apoB in two LDL species with a very different lipid composition. Our results indicate that the conformation of apoB in LDL indeed depends strongly on the physical state of the lipoprotein core, and less on the lipid composition of the core per se.

2. Materials and methods

2.1. Isolation of normal LDL (N-LDL)

N-LDL was isolated from the blood of a healthy, normolipidemic, non-smoking, fasting, 43-year-old male donor. Blood samples were collected in vacutainer tubes containing ethylene diamine tetraacetate (EDTA) and centrifuged for 20 min at 3000×*g* and 4°C. The supernatant plasma was immediately supplemented with gentamicin (5 μg/ml), aprotinin (0.2 U/ml), leupeptin (50 μg/ml), phenyl methylsulfonyl fluoride (175 μg/ml), and NaN_3 (0.02%). N-LDL was isolated from this plasma by sequential floatation ultracentrifugation in a Beckman Ti70 rotor, essentially as described by Lindgren et al. [20]. Purified N-LDL was transferred into 150 mM NaCl, 4 mM EDTA, 0.02% NaN_3, pH 7.4, using a desalting column, and stored under nitrogen at 4°C.

2.2. Isolation of lipoprotein-deficient plasma

Lipoprotein-deficient plasma was obtained using ultracentrifugation, essentially as described by Galeano et al. [21]. Briefly, plasma from a healthy, normolipidemic, non-smoking, fasting, female donor was adjusted to a density of 1.21 g/ml and centrifuged in a Beckman Ti70 rotor for 22 h at 195 000×*g* and 20°C, to float all lipoproteins. Lipoproteins and infranatant were removed from the top of the centrifuge tube and discarded. The remainder of the plasma, containing

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the lipid transfer protein (LTP), was resuspended and dialyzed overnight at 4°C in 150 mM NaCl, 4 mM EDTA, 0.02% NaN_3, pH 8.25.

2.3. Production of triglyceride-rich LDL (T-LDL)

T-LDL was produced by the in vitro exchange of neutral lipids between Intralipid (Sigma Chemical Company, St. Louis, MO, USA) and N-LDL, using a modification of the procedure described by Galeano et al. [21]. Briefly, Intralipid (125 mg triglyceride) and LDL (24 mg total cholesterol) were incubated for 7 h at 37°C with lipoprotein-deficient plasma (1100 mg protein), in a total volume of 21 ml (pH 8.25). T-LDL was isolated from this incubation mixture by sequential floatation ultracentrifugation as described above for the purification of N-LDL.

2.4. Compositional analysis of N-LDL and T-LDL

Protein concentrations were determined by a modified Lowry method, using bovine serum albumin as a standard [22]. Total cholesterol and triglyceride concentrations were determined using Sigma kits 402-20 and 344-20, respectively (Sigma Chemical Company, St. Louis, MO, USA). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to Laemmli [23].

2.5. Differential scanning calorimetry (DSC)

DSC was performed using a MicroCal, model MC2, Differential Scanning Calorimeter (MicroCal Inc., Amherst, MA, USA). Reference saline solution and LDL samples were degassed under vacuum, and 1.5 ml of each were loaded in the reference and sample cell of the calorimeter, respectively. LDL samples were analyzed at a protein concentration of 4–6 mg/ml. Thermograms were recorded over a temperature range of 10–45°C, at a heating rate of 60°C/h. For each analysis, samples were cooled and reheated twice to demonstrate reversibility and reproducibility of the observed phase-transitions.

2.6. Circular dichroism (CD) spectroscopy

Temperature-dependent CD spectroscopy was performed using an Olis RSM 1000 Spectrophotometer (Olis, Bogart, GA, USA). LDL samples were analyzed in 10 mM Na_2PO_4, pH 7.4, at a protein concentration of 0.45 mg/ml. Samples were scanned from 260 nm to 184 nm in a 0.2 mm cuvette. Scans were baseline-corrected and performed in triplicate. Secondary structure content was calculated using Compton fit procedures of the Olis Global Works software package (Olis, Bogart, GA, USA).

3. Results

3.1. DSC of N-LDL and T-LDL

Table 1 shows that the LTP-mediated lipid exchange procedure described in Section 2 increases the triglyceride content of N-LDL at the expense of cholesteryl esters, generating T-LDL particles with a lower total cholesterol/triglyceride ratio. The lipid composition of this T-LDL is very similar to the lipid composition of LDL from hypertriglyceridemic subjects [11]. Earlier studies have established that the triglyceride content of LDL is inversely correlated with the phase-transition temperature of the lipoprotein core [24]. In agreement with this, DSC analysis demonstrates that the cholesteryl ester core of N-LDL has a phase-transition temperature of 27.5 ± 0.4°C, while the core of T-LDL has a phase-transition temperature of 14.3 ± 0.7°C (Fig. 1, Table 1).

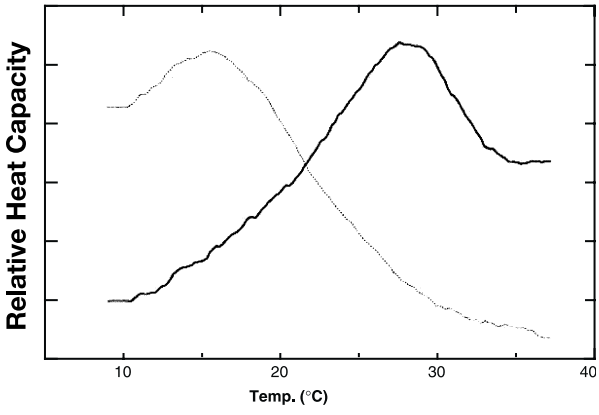

Fig. 1. DSC of N-LDL and T-LDL. Representative thermograms indicate liquid-crystalline to liquid phase-transition temperatures of ~28°C for the core of N-LDL and ~15°C for the core of T-LDL. Similar DSC analyses were performed on three separate N-LDL preparations from the same normolipidemic subject, and on three separate T-LDL preparations. The average phase-transition temperatures of the cores of N-LDL and T-LDL were 27.5 ± 0.4°C and 14.3 ± 0.7°C (means ± S.D.), respectively.

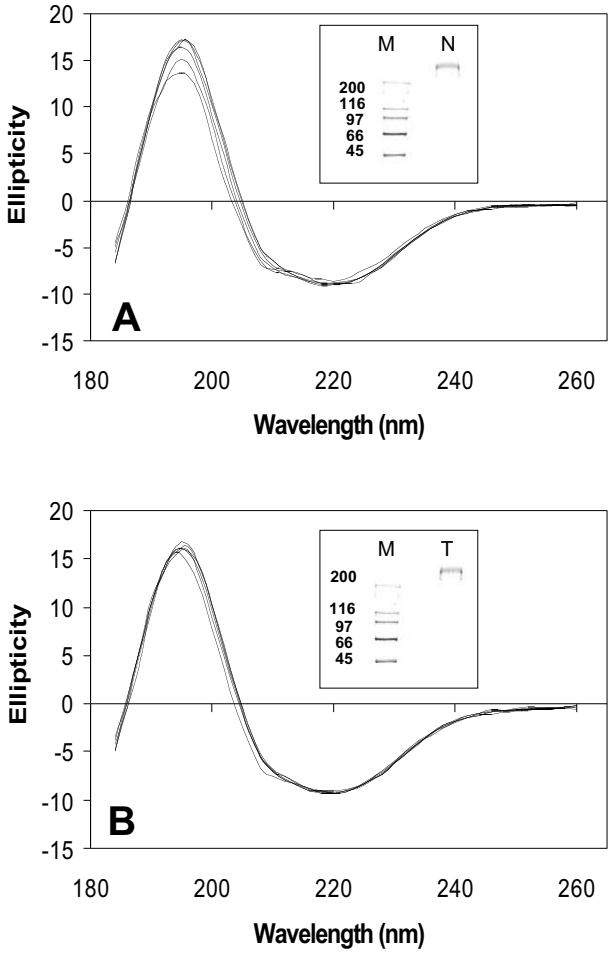

Fig. 2. Typical CD spectra of a single N-LDL sample (A) and a single T-LDL sample (B), recorded at 7, 15, 24, 30, 37, and 45°C. Each of the shown spectra is the average of three scans. Similar temperature-dependent analyses were performed on three separate N-LDL preparations from the same normolipidemic subject, and on three separate T-LDL preparations. Average secondary structures calculated from these analyses are shown in Fig. 3. Insets in A and B show SDS–PAGE analysis of intact apoB in N-LDL (N) and T-LDL (T), respectively. M: molecular weight markers.

3.2. CD spectroscopy of N-LDL and T-LDL

CD spectra of N-LDL and T-LDL, collected at six different temperatures ranging from 7°C to 45°C, are shown in Fig. 2A,B. Using N-LDL, significant temperature-dependent differences in ellipticity are seen in the 210–190 nm wavelength range (Fig. 2A). In T-LDL, this temperature dependency is mostly absent, except for a significant difference between the

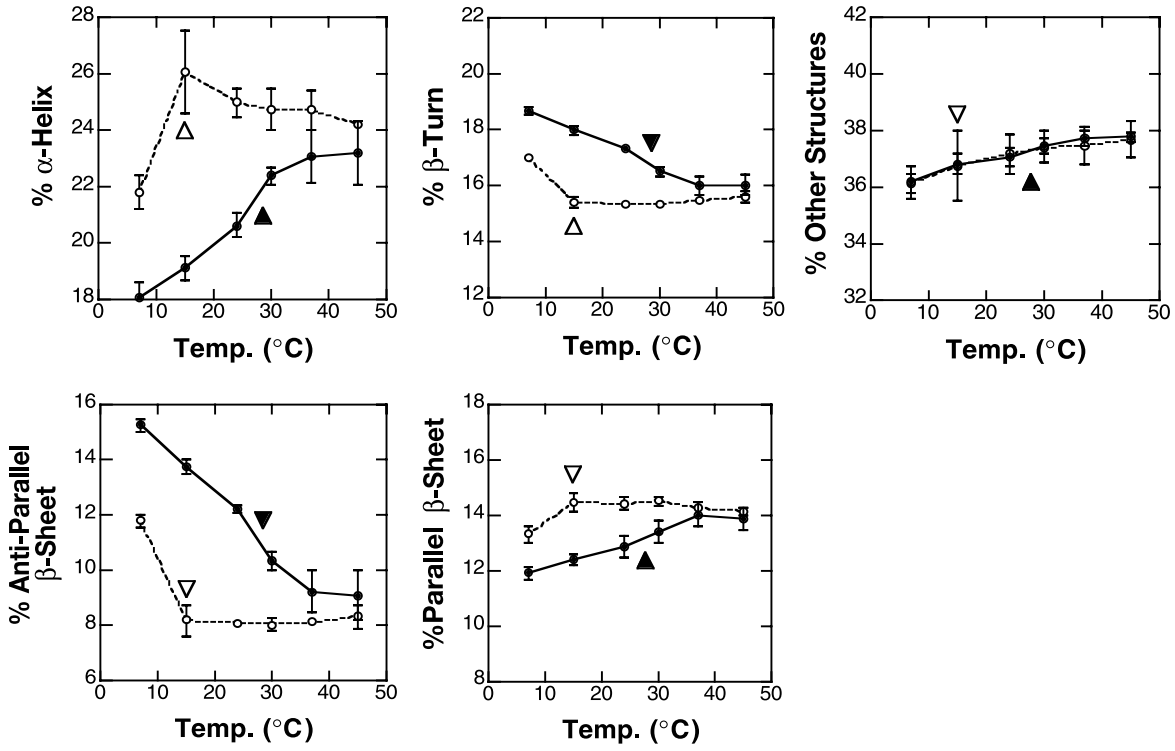

Fig. 3. Secondary structure content of apoB in N-LDL (●) and T-LDL (○) at different temperatures. Presented values are means ± S.D. of three analyses (such as the typical analyses shown in Fig. 2). Closed and open arrowheads indicate phase-transition temperatures of the N-LDL and T-LDL cores, respectively.

spectrum recorded at 7°C and the spectra recorded at the other five temperatures (Fig. 2B). SDS–PAGE of the lipoprotein preparations demonstrated that apoB was intact and the only apolipoprotein component of N-LDL and T-LDL (insets of Fig. 2A,B).

Fig. 3 shows the secondary structure content of apoB in N-LDL and T-LDL at different temperatures, as calculated from the CD spectra of Fig. 2. Fig. 3A demonstrates that gradual heating of N-LDL from 7°C to the phase-transition temperature of the N-LDL core (i.e. ~28°C) gradually increases the α-helical content of apoB from approximately 18 to 23%. Further heating of the preparation from 28°C to 45°C has no additional effect on α-helical content. Similarly, gradual heating of T-LDL from 7°C to the phase-transition temperature of the T-LDL core (i.e. ~15°C) increases the α-helical content of apoB from approximately 22 to 25%, and further heating of the preparation from 15 to 45°C has no significant additional effect on the α-helical content (Fig. 3A). Thus, the α-helical content of N-LDL and T-LDL is very different at 20°C, when N-LDL has a liquid-crystalline core and T-LDL has a liquid core, but very similar at 40–45°C, when both lipoproteins have a liquid core.

Fig. 3B shows an opposite effect of temperature on the anti-parallel β-sheet content of apoB. Gradual heating of N-LDL from 7°C to the phase-transition temperature of the N-LDL core (i.e. ~28°C) decreases the anti-parallel β-sheet content of apoB from approximately 15 to 10%, and further heating of the preparation from 28 to 45°C has no additional effect on anti-parallel β-sheet content (Fig. 3B). Similarly, gradual heating of T-LDL from 7°C to the phase-transition temperature of the T-LDL core (i.e. ~15°C) decreases the anti-parallel β-sheet content of apoB from approximately 12 to 8%, and further heating of the preparation from 15 to 45°C has no additional effect on anti-parallel β-sheet content (Fig. 3B). Thus, the anti-parallel β-sheet content of N-LDL and T-LDL is very different at 20°C, when N-LDL has a liquid-crystalline core and T-LDL has a liquid core, but very similar at 40–45°C, when both lipoproteins have a liquid core.

Similar temperature-dependent trends are seen in the parallel β-sheet and β-turn content of apoB, for both N-LDL and T-LDL, but the effect of temperature on these structures is less pronounced than the effect on α-helical and anti-parallel β-sheet content of apoB (Fig. 3C,D). There is no observable difference between N-LDL and T-LDL in the content of 'other' (i.e. random coil) structures at any of the indicated temperatures, and the percentage random coil structures remains fairly constant across the 7–45°C temperature range (Fig. 3E).

Table 1
Lipid composition and core phase-transition temperature of N-LDL and T-LDL

LDL	Chol/protein	TG/protein	Chol/TG	T_m
N-LDL	1.42 ± 0.10	0.19 ± 0.03	7.50 ± 0.56	27.5 ± 0.4°C
T-LDL	1.26 ± 0.09	0.39 ± 0.06	3.29 ± 0.25	14.3 ± 0.7°C

Compositional values are w/w ratios. Chol: total cholesterol (free cholesterol plus cholesteryl ester); TG: triglyceride; T_m: midpoint of the phase-transition of the lipoprotein core. Values are averages of three determinations ± S.D.

4. Discussion

The present study shows a correlation between changes in the physical state of the LDL core and changes in the secondary structure of apoB. These results are in agreement with earlier infrared spectroscopic studies of Bañuelos et al. [25], who determined the secondary structure content of apoB in N-LDL at two different temperatures. However, instead of comparing the structure of apoB at two temperatures, our current data show gradual changes in the secondary structure of apoB at multiple temperatures below the phase-transition temperature of the N-LDL core, and reveal a lack of temperature-dependent change above the phase-transition temperature of the core. In addition, we extended our observations to in vitro-produced T-LDL, which has a core with a lower phase-transition temperature. The influence of temperature on the secondary structure of apoB, as observed in N-LDL samples, is also seen in T-LDL samples, however, in T-LDL all temperature-induced effects are shifted downward on the temperature scale, along with the downward shift in the phase-transition temperature of the core from 28°C to 15°C. As a result, the secondary structure contents of apoB in N-LDL and T-LDL are very different at 20°C, when T-LDL has a liquid core and N-LDL has a liquid-crystalline core, but very similar at 37°C, when both cores are in the liquid state (Fig. 3). These results indicate that the secondary structure of apoB in LDL strongly depends on the physical state of the lipoprotein core, and less on the lipid composition of the core per se.

Our results have implications in at least two different areas of interest. Firstly, although no high-resolution structures of LDL have been obtained to date, X-ray crystallographic [26–28] and cryo-electron microscopic [14–17] analyses of the lipoprotein particle have been most successful with samples prepared at low temperatures, i.e. below the phase-transition temperature of the LDL core. The present study cautions that information obtained from these structural studies shows apoB in a non-native conformation, and that future efforts should include analyses of LDL particles with a liquid core. Secondly, studies with miniature swine [29] and non-human primates [30–32] have shown that diets with a high content of cholesterol and saturated fat may elevate the phase-transition temperature of the LDL core above body temperature. Consequently, these diets may generate LDL particles that have a liquid-crystalline core in vivo. Our results indicate that diet-induced changes in the physical state of the LDL core may affect the in vivo conformation of apoB on the LDL surface. This conformational change may influence the metabolic and pathogenic properties of LDL, and may in part be responsible for the atherogenicity of diets with a high content of cholesterol and saturated fat.

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